

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Re Application of: Lal *et al.*

Art Unit: 1645

Application No. 09/763,397

Filed: February 16, 2001

For: RECOMBINANT MULTIVALENT MALARIAL
VACCINE AGAINST PLASMODIUM
FALCIPARUM

Examiner: Vanessa L. Ford

Date: December 20, 2002

CERTIFICATE OF MAILING

I hereby certify that this paper and the documents referred to as being attached or enclosed herewith are being deposited with the United States Postal Service on Feb 6, 2003 as First Class Mail in an envelope addressed to: COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231.

William D Noonan

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COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SECOND DECLARATION UNDER 37 C.F.R. § 1.131

I, Ya Ping Shi, hereby declare as follows:

1. I am a co-inventor of the subject matter described and claimed by the patent application referenced above, *i.e.*, United States application No. 09/763,397 (hereafter the '397 application). I currently am employed by the Centers for Disease Control and Prevention (CDC), the assignee of the '397 application, which is located in Atlanta, Georgia. I was employed by the CDC while developing the invention described and claimed in the referenced application.

2. I understand that claims pending in the present application have been rejected in view of Tine *et al.*, *Infection and Immunity*, 64(9): 3833-3844, 1996. I understand that Tine *et al.* has been cited as allegedly anticipating certain claims pending in the referenced application, or, in the alternative, as allegedly rendering the claimed embodiments obvious.

3. The publication date of Tine *et al.* is September 1996. United States Provisional Application No. 60/097,703 was filed on August 21, 1998. However, the co-inventors named on the '397 application invented the subject matter covered by the claims pending in the '397 application prior to the September 1996 date that Tine *et al.* became available as a reference.

4. I previously executed a first Declaration under 37 C.F.R. § 131, including the attached Exhibits A and B, in connection with Applicant's June 11, 2002 amendment and response. Exhibit A consists of true and accurate facsimile photocopies of 21 corresponding pages from my laboratory research notebook. Exhibit B consists of one page of CDC Biotechnology Core Facility Records, showing my request for oligonucleotide synthesis, and the sequences of the requested oligonucleotides. This request was made prior to September 1996. These oligonucleotides were used in the reduction to practice of the invention, as described in Applicant's June 11, 2002 amendment and response. The contents of these pages of Exhibits A and B, and pertinent statements made on these pages are discussed in detail in Applicant's June 11, 2002 amendment and response.

5. Exhibits A and B were previously submitted as evidence that the conception and reduction to practice of the invention recited in the claims of the '397 application occurred in the United States of America prior to November 1997, the effective date of the Gilbert *et al.* publication cited as allegedly anticipating prior art in the Office action mailed February 11, 2002. As noted on my previous Declaration, all dates stated on Exhibits A and B were redacted prior to submission, but were made prior to November 1997, the effective date of the Gilbert *et al.* publication.

6. Similarly, all dates stated on Exhibits A and B were prior to September 1996, the effective date of the Tine *et al.* publication.

7. All statements made herein and of my own knowledge are true and all statements made on information are believed to be true. Furthermore, these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements made may jeopardize the validity of the application or any patent issuing thereon.

Ya Ping Shi

Ya Ping Shi, Ph.D.

1/28/2003

Date



EXHIBIT A

First PCR

AA: GTC - GTC
BB: G3 - G6
CC: G7 - G12

7-11
52-56
57-61

94°C 5min.
94°C 45"
45°C 1min.
72°C 1.5min. 8 cycle (p139)

500ng/each 73.5

AA: 2x4 = 8ul 65.5

BB 2x4 = 8ul 65.5

CC 2x6 = 12ul 61.5

16ul dNTP
10ul Buffer
0.5ul Taq
26.5ul.

Second PCR.

53.5

16ul dNTP
10ul buffer
0.5ul Enz
Total 0.182
46.5

94°C 5min.

94°C 45"
45°C 1min
72°C 1.5min. 25 cycle (p141)

AA: DD1 1ul 52.5+5+5
DD2 2.5ul 51+5+5 → G0
DD3 5ul 48.5+5+5 → G2 ✓
DD4 10ul 43.5+5+5
BB: EE1 1ul 52.5+5
EE2 2.5ul 51+5 → G3 ✓
EE3 5ul 48.5+5 → G6 ✓
EE4 10ul 43.5+5

C: FF1 1ul 52.5+5
FF2 2.5ul 51+5 → G7 ✓
FF3 5ul 48.5+5 → G12
FF4 10ul 43.5+5

Redo CC₀: G7 - G12 = 12ul

dNTP	16ul	c.; Tag
10x Buffer	10ul	
H ₂ O	61.5ul	
	<u>100ul</u>	

94°C 5min
 94°C 45"
 40°C 1min
 72°C 2min

} 8 cycle

CC₁: G7 - G8 (only do second PCR) = 4ul + 69.5ul H₂O
 CC₂: G9 - G12 2x4 = 8ul + H₂O 65.5

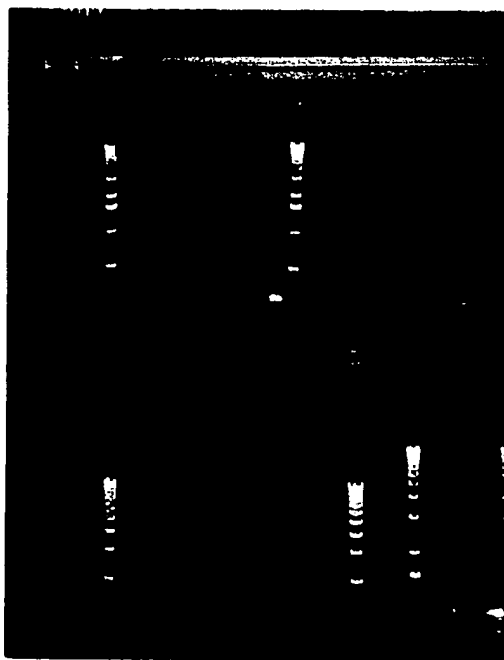
Do SOE: G6 - G6

	DD ₁ + EE ₁	H ₂ O	16ul dNTP
GG ₁	1ul + 1ul = 2ul	61.5	63.5 10ul Buffer
GG ₂	2.5ul + 2.1ul = 5ul	58.5	5ul G0
GG ₃	5ul + 5ul = 10ul	53.5	5ul G6
GG ₄	10ul + 10ul = 20ul	43.4	0.5 Tag
			<u>36.5</u>

program 141

FF ₁	} CC ₁	primers	67.5	H ₂ O	16ul dNTP
FF ₂		G7	1ul	62.5	10ul Buffer
FF ₃		G12	2.5ul	61	oligos 10ul
FF ₄			5ul	58.5	Tag 0.5
FF ₅	} CC ₂	primers	53.5		<u>36.5</u>
FF ₆		G9	1ul	94°C 5min	
FF ₇		G12	2.5ul	94°C 45"	
FF ₈			5ul	40°C 1min	
				72°C 2min	cycles #41

Result: GG-4



FF-3 did not work probably because of 1

prepare new temp 0110 GG-4 also AL1065

Redo: $CC'_2 \rightarrow CC''_2$ and CC''_3 .

3.5

CC''_2 G9 G10 G11 G12 $\times 2 = 8ul$. 65.5

CC''_3 G9 G10 G11 AL1064 $\times 2 = 8ul$. 65.5

\times
works
well

16ul dNTP.
10ul Buffer.
0.5 Tag

Same to before.

Second PCR.

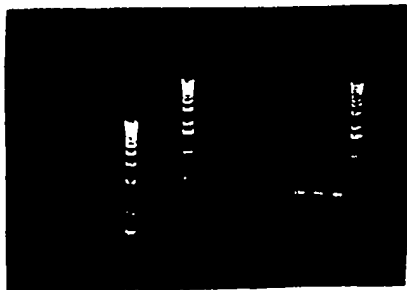
FF''₁
FF''₂
FF''₃
FF''₄ } CC''_2 primers
G9
G12

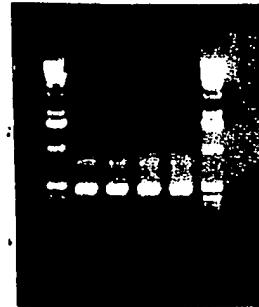
1-20
1ul 62.5
2.5ul 61
5ul 58.5
10ul 53.5

16ul dNTP
10ul buffer
c1180 10ul
Tag 0.5ul.

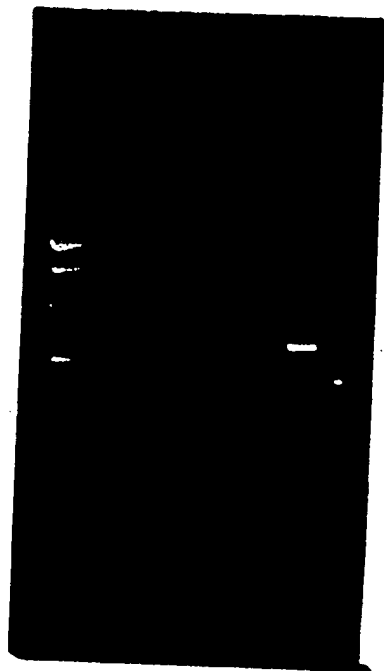
FF''₅
FF''₆
FF''₇
FF''₈ } CC''_3 G9
AL1064
works well

Same to before.
1141





114



11



Set for G₁₇ - G₁₁ + AL-1065

	CC ₁	FF ₁	H ₂₀	63.5	16ml dNTP
HH ₁	1ul	+ 1ul	61.5		10ul buffer
HH ₂	2.5ul	+ 2.5ul	58.5		5ul G ₁₇
HH ₃	5ul	+ 5ul	53.5		5ul AL-1065
HH ₄	10ul	+ 10ul	43.5		0.5 Tag

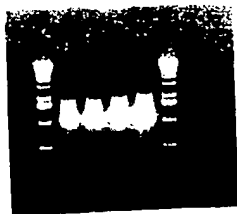
36.5

program #41

	G ₁₇	+ HH ₁	H ₂₀	63.5	16ml dNTP
II ₁	1ul	+ 1ul	61.5		10ul buffer
II ₂	2.5ul	+ 2.5ul	58.5		5ul AL-1065
II ₃	5ul	+ 5ul	53.5		5ul AL-1065
II ₄	10ul	+ 10ul	43.5		0.5 Tag

36.5

program #41



good!

Further cleaning and cloning,
sequencing.

A: Run gel and cut ~~and~~ and clean.

① gene clean (from product of PCR)

② gel clean through column (according introduction of manufacturer) (50ul of PCR product tube one is pellet (store in -20°C)

another ~~is~~ has 20ul water. From this, 10ul of was take for digestion.

B. digestion:

Not I : 26ul water.
3ul Buffer
1ul Not I

II, Gene clean
II, column clean

1h 37°C

pellet.

BamHI

26ul H₂O
3ul buffer
1ul BamHI

1h 37°C

Ligation.

Water 13ul
Vector 1ul (BamHI and Not I digest)
5x lig buffer 4ul
T4 ligase 2ul

Control I Control II
" 15ul
" "
" "
" x

over night (4°C)

NotI digestion:

Vector:

10ul
3ul
3ul
4ul
10ul

Vector (concent 3.2ug/ul)

10x buffer

BSA

NotI

H₂O

30ul 37°C 1.5h

target

II₂ and control (MSP-1)

2ul H₂O

3ul BSA

3ul 10x Buffer

2ul Enzyme

30ul 37°C 1.5h

BamHI digestion

Vector

BamHI

Buffer

water

4ul

3ul

23

30ul

37°C 1.5h

BamHI

Buffer

water

2ul

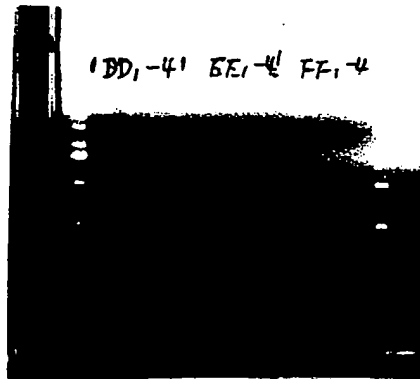
3ul

25ul

30ul

37°C 1.5h

Result



BD₁-4 BE₁-4 FF₁-4

FF₁-4 did not work because first PCR (CC) annealing temp was too high

Need redo CC (first PCR), then FF₁-FF₄

ligation as before
transformation as before

result. not so much white clones. probably vector
was not properly digested.
Chunfu further purify vector.

pick up 40 clones grow overnight.

cell PCR: as regular. 10 μ l cell 94°C 5min.

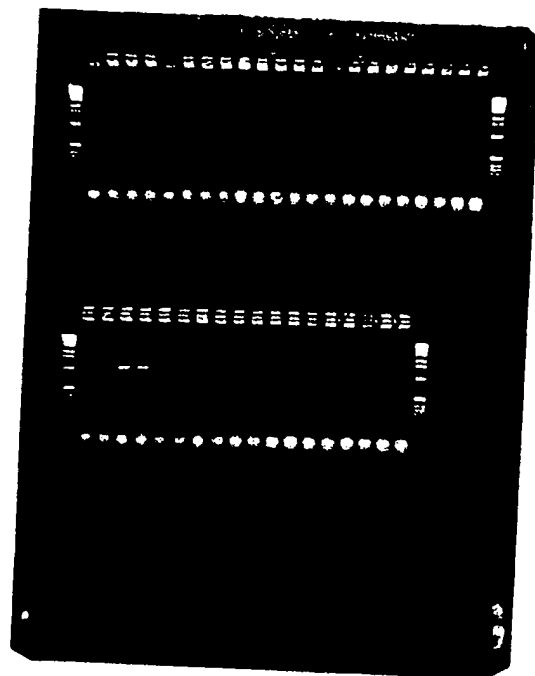
Eligo	AL1064	2.5 μ l
	AB1065	2.5 μ l
	Buffer	5 μ l
	dWTP	8 μ l
	Taq	0.5
	1+20	27.75
		<hr/> 40 μ l

15 cycle 94°C 45" 50°C 45" 72°C 60"

primers clone

1, 2, 3, 4, 6, 8, 17, 21, 22,
25, 26, 27, 31, 33, 36, 39, 40,

5' & 3' back



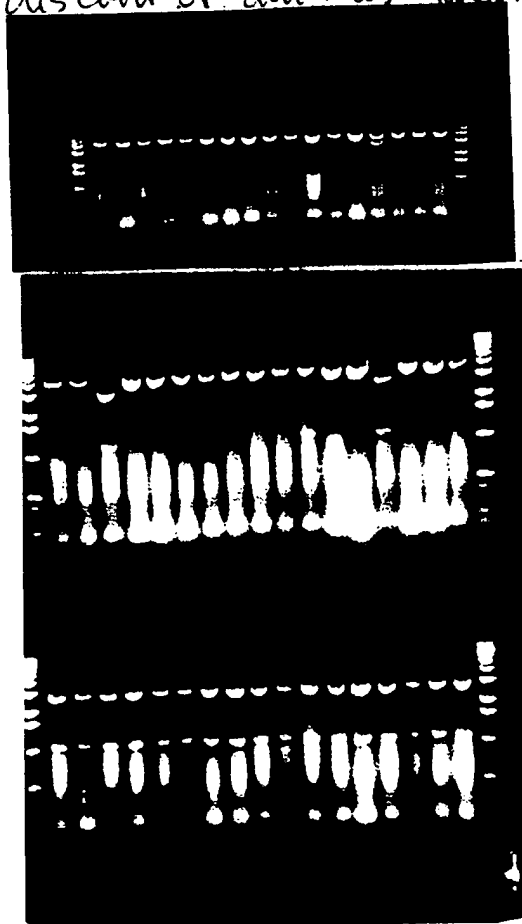
Save cell
Savulas SGV R# SGV-H11111 Gene Vaccine
Fusion to sign

digest all positive (17) clones (based on PCR)

Single digestion: BamHI or NotI

double digestion BamHI and NotI.

Result: Clone 3, 26, 33 are not pure clones.
discard or don't use them



2, 6

Plasmid pBacPAK8 and pBacPAK9 (from Sayo)
218/100ul 218/100ul

Transformation:

100ul plasmide (200ng)

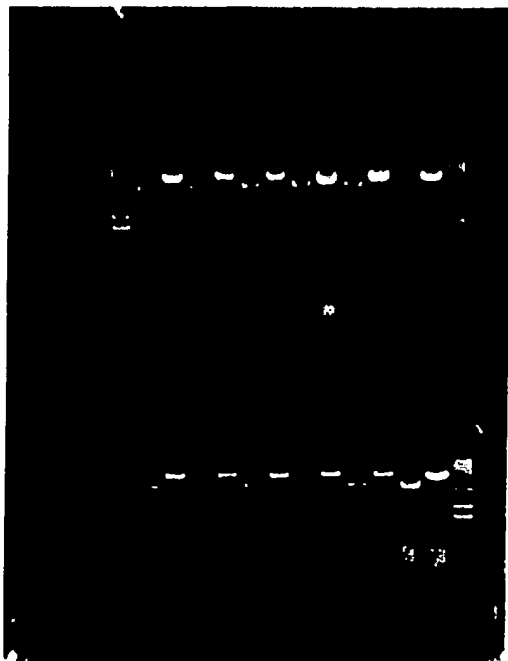
100ul XL-blue cell

procedure as regular.

plating overnight
growth well

Miniprep of pBacPAK8 and pBacPAK9 -

run undigested and digested plasmid



100ng/ul $\times 19 =$

1.9ul

5-1-19

This result confirm that. ~~no~~ orders are
no problem. also confirm (11) (20) (63) clones
are true clones:



Will sequence clone 20.

Methylation:

Clone 63 Vector correct.

Clone 20 Most target correct.

Clone 63 methylation.

Reaction:	3ul	Taqi methylation
	3ul	NEB 4 Buffer
	0.3ul	BSA
	22.2ul	H ₂ O
	1.5ul	Mix SAM

1 hr 65°C

Mix: 5ul NEB 4 Buffer + 45ul H₂O + 1.25ul SAM

0.6ul Nac. (SM)

60ul Ethanol (100%)

Hind II cut

clone 63 (two pieces very big)

clone 20 (more pieces
vector small)

run gel

standard

20

63

standard

(more small) (+ a = big)

restriction conditions:

3 ul	buffer
8 ul	Hind ^{III}
21 ul	1420

1.5h 37°C

Result:

clone 20

clone 63

|||||

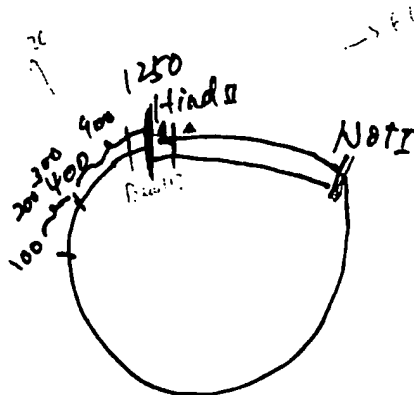
—

Δ

Δ 1kb
— 0.9kb

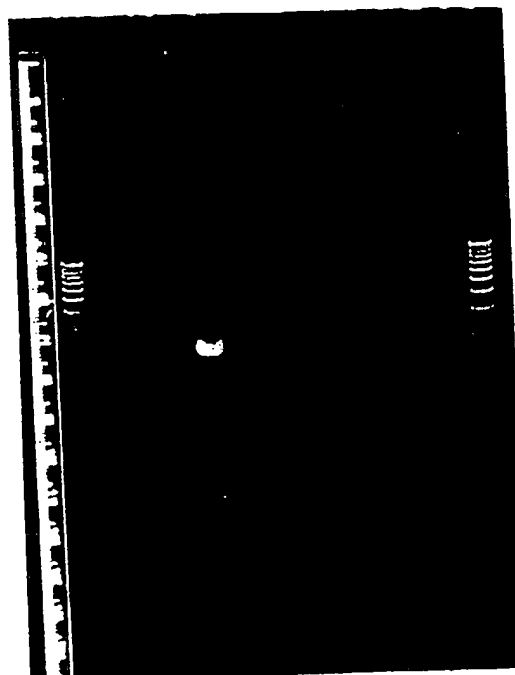
— — 1kb

— 0.3 kb



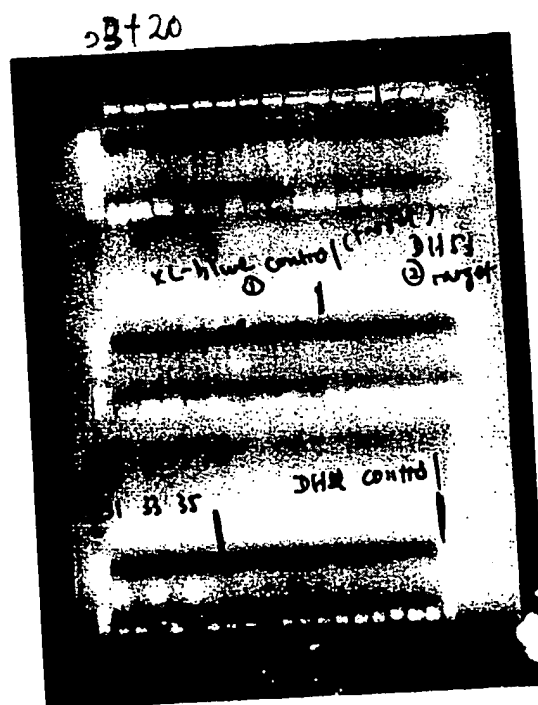
Δ cut fragment

1944



clone 63 + 20 ligation see before

clone PCR primer: AL1097
AL1064



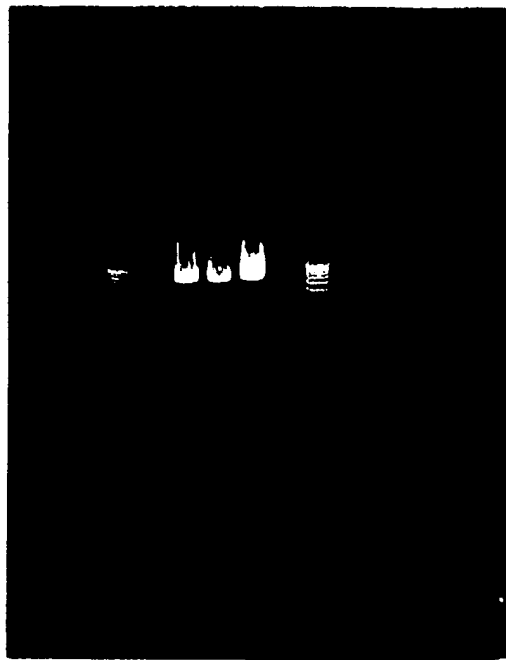
clones 21, 31, 33, 35
are positive

same as name:

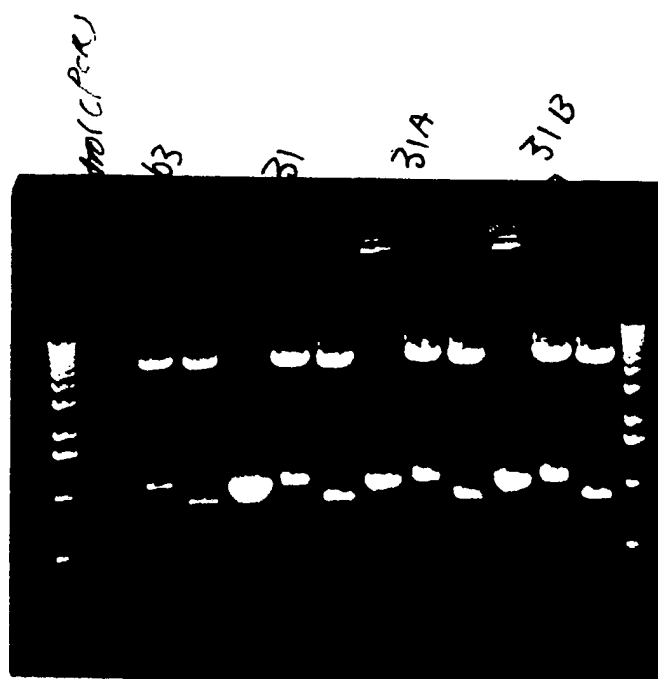
~~38 FTS/CL-DT21/63+20~~

Pac8/63+20/number ^{number}

Banc 17. digestion: 63+20 (21, 31, 33, 35) 63



restriction sequencing methylation



Save clone 31A and 31B
named as pac8/63+20/31A and 31B

Date	Oligo ID	DNA Syn No.	Sequence	Length-mer
	G0	953882	GTCCGGATCCATGAAATTTCTAGTCAACGTTGCCCTTGT TTTATGGTCGTGTACATTTCTTACATCTATGCCGGalcalcalca	94
	GL	953883	tcatacalcal ttalcctcalcttacccticalgalgalgalgalgalgal	40
	G1	953884	gaaggtlaaagaatgaagataaagAGAGATGGAATTAACGAAGACAAAC GAGAAATTAAAGGAACCAAAACATATAAAATTAAGCAAC CAGGGGATGGTAAATCcttggtcccatgtagtga	120
	G2	953885	lattaagacataaacaacCTGGAACATTTTCCATTTTACAAATTT TTTTTCAATATCATTTTTCATTAATCTAATTCGTCTTAGGTT TTCACACAAGTtaccatacalgggaccaag	120
	G3	953886	TGTTGTTAATGTCGTAATAGTAATTCGTGATGTTTCAGA CAATTAGATGAAGAGAGAGAAATGTAATGTTTATTAGAAAG ATTGAGGTAGCAACGGAAGAAATCACAATGTGAATGTAC	120
	G4	953887	tggtatcttcaaatccatCCATCAGGATTTGCATTTGGGTTTGGCT TTGGGTTTGCATTTGAATTTGTATATTGCACAATAGGCTT AGAAATCAGGTTTAgtaacatacalgtgatttt	120
	G5	953888	gaaatgtaagataaccaCATGTAATGAATTTTCAGCAATTGAT CTTGAAATGCTGAAATAATATGATAAAATGGATGAACCAC AACATTATGGGAATcaccctccatggaag	120
	G6	953889	gcattgtttagaaltggttGCTTAGTTGATTAACCTATAAATTTAG AATTGCTTTTATATACTGATACAAACCTTTATCATTTGGTT TATAATAATcttctaalgagtgagtgat	120
	G7	953890	aacacatcatalaaacaatgctGGACAAACATGGACATATGCATGGTA ACGAGAGGGAAAGATGAGAGAACGCTTACTAAGGAATATG AAGATATTGTTTGAaagagttaccatalatgata	120
	G8	953891	cttaatttttcaataicGTTAAATGTTGTTCAATATTGTTAGGTTGA TCACTTTTTTGATATGATGTTCCCAATTAATCTGTCTCT TCCAAAGTTtalcatalatgtaactctt	120
	G9	953892	agattatgaataaataaagAAGGTAAGCCCTTGATAAATTTGGA AATATCTATGATTATCACATATGAGCAATTCATGCCATCTAG TACAAAGTCATCAagtcacataaaltgtaaaic	120
	G10	953893	atccttatatttgcataaaacCTTTTCATATAACCTATACCGAAGAA ATCTCTGATTTTCAGCTTTAAATTTTTTTCATTAATCTTTGTAG CTAGACTagcigtatttcaattgtagact	120
	G11	953894	tttagcgaataatlaagatGATTAGAAAGTTAGTATCAGAGAGATA TTGTTGCAAGAAATCAAAATAGATATAACTAATTTTCTTAttgaa laagcggccgcccgaac	104
	G12	953895	gtcggcgccgcctatcaaa	20